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Nutritional and Microbiological Composition of Serra Estrela Cheese: A Paradigm to Overcome

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Abstract

Background: Milk and dairy products are an excellent source of well-balanced nutrients with multiple uses as a snack, dessert or food ingredient. Serra Estrela cheese, a traditional variety manufactured in the centre region of Portugal, is part of the mankind´s ancient cultural heritage, made from raw sheep milk it is assumed as an iconic gourmet cheese, when compared with other Portuguese cheeses. This work intended to monitor the manufacturing process along the period of production, to evaluate the factors that are decisive for the reproducibility of Serra Estrela cheese in geographical and temporal terms, as well as to achieve the knowledge of the Serra Estrela cheese lipid nutritional characteristics.

Methods: Twenty-four Serra Estrela cheese samples originating from representative cheese producers of the region were analysed for their nutritional characteristics, such as moisture, fat, protein and salt, using the FT-NIR (fourier-transform near infrared spectroscopy) technique as an expeditious method. A gas chromatography with flame ionization detection (GC-FID) was used to perform the lipid fraction study. Microbiological analyses were performed for *Escherichia coli* and coagulase-positive Staphylococcus. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS version 23.0). The level of significance was accessed by Tukey's test considering p-value < 0.05 to be significant.

Results: In the cheeses studied, the moisture varied from 46 % to 52 %, the butyric content from 19 to 30 %, the protein content between 19 and 24 % and the salt from 0.9 to 1.8 %. The determinations of *Escherichia coli* and coagulase-positive Staphylococcus in some Serra Estrela cheeses exceeded the maximum allowed values per law in Portugal. Regarding to the fatty acids profile, there was no obvious tendency observed, but was found a significative amount of short and medium chain saturated fatty acids, as well as polyunsaturated fatty acids.

Conclusion: Considering that year 2017 was considered an exceptional year, both in terms of the severe drought and the fires that devastated the region, with repercussions on health and animal production, it is reasonable that some cheeses exceeded the maximum permissible levels of microorganisms. Although there are significantly differences between the total fat and protein content of Serra Estrela cheeses from different producers, relatively to polyunsaturated, ω-3 and ω-6 fatty acids fractions, all cheese producers are very homogeneous. This work was extremely important for an initial evaluation of the Portuguese Serra Estrela cheese production in 17/18 and for the design of future experimental work.

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Background

Artisanal production of regional cheeses is a part of the gastronomic and sociocultural heritage of many countries in Europe, including Portugal. World consumption of fresh dairy products and processed dairy products is poised to grow annually by 2.1 % p.a. and 1.7 % p.a. respectively, over the next decade. The largest share of milk and dairy product consumption is in the form of fresh dairy products, taking up about 50 % of the world's total milk production. With an annual output, on average, of 96.9 metric tons of cheese, and an average value of 1.46 million Euros (€) this local economic activity is very important for regional development [1]. Consumption dynamics differ considerably between developed and developing countries. Developed countries consume primarily processed milk products, with per capita consumption of cheese growing at 0.9 % p.a. [2].

Undernutrition can be acute or chronic in form. Chronic undernutrition results from a long term or recurrent inadequate diet and/or other co-morbidities (such as infectious diseases). In children, chronic undernutrition manifests itself in a linear growth deficit, Serra Estrela (SE) cheese is the most traditional cheese manufactured in Portugal. It acquired the Protected Denomination of Origin (PDO) which included several aspects that make it unique: i) its manufacture that encompasses coagulation of raw sheep milk using the wild thistle flower of *Cynara cardunculus* L. (without addictions of any starter or non-starter cultures) and ii) its final buttery texture and typical flavour [3]. The peculiar aroma compounds of Serra Estrela cheese result partially from the action of indigenous microorganisms and enzymes on sugars, lactose, glycerides and free amino acids [4]. This, combined with the enzymatic reactions occurring during ripening, contribute to the ultimate unique flavour profile of this matrix. It is a fatty blocked shape soft product weighing between 1.00-1.60 kg per piece.

The milk of small ruminants such as sheep is of economic interest in certain areas of Portugal. Sheep milk is mainly used to produce cheese, yogurt, and whey cheeses. It contains high levels of protein, fat, and calcium by casein unit, without presenting high production due to seasonality [5]. Market competitiveness implies as fundamental the valorisation and the differentiation of Serra Estrela cheese components but luckily the demand for these cheeses is very high, especially in Christmas time in Portugal, but it becomes very difficult to reconcile a traditional production method with high competitive industrialized methods and achieve better production numbers.

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Cheese is a nutritious and versatile dairy food that contains a high concentration of essential nutrients relative to its energy content. Cheese fat composition is a paramount factor in the nutritional quality of cheeses; it varies along the season [6], depending on lactation, nutrition [7] and animals breed [8]. Fortunately the use of dairy products is also associated with beneficial health effects beyond pure nutritional value. Dairy has been a rich source with nutraceutical properties used for dietary supplements [9,10]. SE cheese is a good source of omega-3 fats containing about 88% of the RDI of omega-3 fat [11] with a good ratio of anti-inflammatory omega-3 and pro-inflammatory omega-6 fatty acids. Omega-3s are unsaturated essential fatty acids that have a protective effect against cardiovascular diseases (CVD) and some evidence for possible beneficial roles of ω-3 fatty acids for immune function and inflammatory diseases such as arthritis. They also play a role in the proper functioning of the nervous system, specifically the brain [12].

Enzymatic hydrolysis (lipolysis) of triacylglycerols to free fatty acids (FA), glycerol and mono and diglycerides becomes essential for flavour development in cheese [13]. FA of total carbon atom numbers from 1 to 6 are usually classified as short-chain fatty acids (SCFAs), whereas those of 7 to 12 carbon atoms are defined as medium-chain fatty acids (MCFAs). The odour of the first members is pungent, whereas that of the higher members is rancid or none [14]. Both are connected to the cell and all body functions. Along with their function as "fuels" for the oxidative generation of ATP, SCFAs and MCFAs supply anabolic pathways (gluconeogenesis and lipogenesis) with carbon-containing precursor molecules and contribute to the regulation of cell metabolism by triggering signalling pathways. Thus, MCFAs and, in particular, SCFAs play an important role in a proper balance between lipogenesis and oxidative degradation of fatty acids [14]. Capric and caprylic acids, and other MCFAs, have been used for treatment of patients suffering various problems of malabsorption, pancreatic insufficiency or the deficit or absence of bile salts, as well as those subjected to intestinal resection [8]. They also exhibit various regulatory and signalling functions [14]. These MCFAs have also been used in diets of undernourished patients, premature infants, and those suffering from infant epilepsy and other pathologies, because of the great energy giving facility of these compounds [15]. Richards et al. (2016) highlighted the paper of SCFAs in restoring the imbalances in lipid and glucose metabolism, making a contribution to the prevention and treatment of cardiovascular diseases [16]. SCFAs are the key metabolites that connect dietary fibre and gut microbiota to the intestinal health, mediating the suppression of inflammation [17] and carcinogenesis in gut and other organs [18]. Butyric acid plays several beneficial roles in the gastrointestinal tract homeostasis because its easily absorbed by enteric cells, being an important regulator of colonocyte proliferation and apoptosis, gastrointestinal tract motility and bacterial microflora composition [19] as well as in regulating the skin immune system [20]. Dietary guidelines recommend that the amounts of saturated FA in dairy fat, especially C12:0, C14:0, and C16:0, should be reduced because of their potential hypercholesteraemic effects and that this should be done concurrently with an increase in polyunsaturated FAs [21]. From the available information, it can be concluded that the nature of fat content of sheep and goat milk, in comparison with cow milk, presents advantages for consumer health. It is known that metabolic discrimination between varying fatty acids begins in the GI tract, with MCFAs being absorbed more efficiently than long chain fatty acids (LFCAs) [22]. Subsequently, MCFAs are transported in the portal blood directly to the liver and LCFAs are incorporated into chylomicrons and transported through lymph. These structure base differences continue through the processes of fat utilization; MCFAs enter the mitochondria independently of the carnitine transport system and undergo preferential oxidation. These differences in metabolic handling may support that MCFAs hold potential as weight loss agents with unique nutritional and physiologic properties [23].

Literature demonstrations state that there is a negative evidence association between the consumption of different types of cheese (fresh and processed cheese) and excess of weight [24-26]. However, the message that all saturated fatty acids may constitute a problem in dairy products lacks appropriate scientific evidence, and on the contrary, some works can prove divergent results suggesting their importance in many physiological actions, according to the new trends of nutrition, the approach so-called functional nutrition. In fact, Sacks et al. presented a study [27] of 811 overweight people having one of four diets with a range of fat, protein, and carbohydrate compositions, that were successful in causing weight loss. When non-nutritional influences were minimized, the specific macronutrient content used was of minor importance in diets that were successful in causing weight loss.

Although Serra Estrela cheese production is very ancient, there are few studies currently available on its analytical and consequent nutritious composition. In this work, characterization of fat of SE was intended to monitor the manufacturing process along the months of production, to evaluate the factors that are decisive for the reproducibility of SE cheese in geographical and temporal terms, as well as to achieve the knowledge of the SE cheese lipid nutritional characteristics. With the approach undertaken, we are also promoting future work with the broader evaluation of the multifactorial causes that contribute to the diversity of organoleptic and yield characteristics of Serra Estrela cheese, as well as to the chemical analysis of the SE cheese.

Materials and Methods

Twenty-four SE cheese samples originating from 6 representative cheese producers of the PDO region, with 45 days of maturation, were collected in refrigerated boxes and analysed immediately for their nutritional characteristics. Moisture, total protein, total fat and NaCl content were determined through the FT-NIR (fourier-transform near infrared spectroscopy) technique using a NIR Master™ 500 FT-NIR standalone spectrometer. All the samples were analysed in triplicate. A gas chromatography with flame ionization detection (GC-FID) was used to perform the lipid fraction study. The samples were analysed in duplicate. Regarding to the microbiological determinations in SE cheeses, the preparation of the samples was carried out in accordance with ISO 6887-1: 2017 and decimal dilutions were performed according to the technique described in NP 3005: 1985. The determinations of *Escherichia coli* and Staphylococci, followed ISO 16649-2:2001 and ISO 6888-1:1999, respectively. The results were expressed in CFU/g of cheese. The data was analysed using Statistical Package for Social Sciences (SPSS version 23.0). The level of significance was accessed by Tukey's test considering p-value < 0.05 to be significant.

Results and Discussion

Medium values of major constituents of SE cheese determined by FT-NIR are presented in table 1. In the samples studied, the moisture varied between 46 and 52 %, the butyric content between 19 and 30 %, the protein content between 19 and 24 % and the salt between 0.9 and 1.8 % (Table 2). Fox et al. (2016) [28] present similar contents with medium values of 48.7 % of moisture, 27.5 % of butyric content, 21.3

% of total protein and 1.9 % of salt. Carocho et al. [29], Ramos and Juarez [30] and Associação Portuguesa de Nutrição (2018) [11] obtained similar results.

	FT-NIR parameters (%)			
	Humidity	Total fat	Total protein	Salt
SE cheese	48.91±3.22	23.84±4.45	21.96±2.30	1.18±0.36

Table 1: Major components determined by FT-NIR in 24 samples of Serra Estrela cheese.

Values presented as mean ± standard deviation; FT-NIR: near infrared spectroscopy; SE: Serra Estrela.

SE cheese producers	FT-NIR parameters (%)			
	Humidity	Total fat	Total protein	Salt
Q1	45.77±2.24^{a}	28.88±2.03^{d}	19.75±0.65^{a}	1.13±0.13^{bc}
Q2	46.90±0.97^{ab}	29.64±0.44^{d}	19.00±0.30^{a}	0.90±0.09^{a}
Q3	51.87±1.04^{c}	21.85±1.08^{b}	21.29±1.50^{b}	0.96±0.24^{abc}
Q4	47.19±2.07^{ab}	25.63±1.57^{c}	22.58±1.31^{bc}	0.96±0.19^{ab}
Q5	49.03±3.45^{b}	20.46±2.80^{ab}	24.48±1.83^{d}	1.77±0.27^{d}
Q6	52.21±1.16^{c}	19.00±0.99^{a}	23.18±1.31^{cd}	1.18±0.36^{c}

Table 2: Major components determined by FT-NIR in 24 samples of Serra Estrela cheese, by cheese producer.

Values presented as mean ± standard deviation; for each parameter, values with different letters are significantly different (Tukey, $p < 0.05$); FT-NIR: near infrared spectroscopy technology; SE: Serra Estrela; Q1-Q6: Serra Estrela cheese producers.

Also, we can verify that there are significant differences between cheeses of different producers (Table 2). Samples from cheese producers Q1 and Q2 presented significantly higher values of fat and significantly lower values of protein, and the cheese samples from Q5 and Q6 show the opposite trend. The Q3 cheese producer presented the most balanced values in terms of fat and protein content with 21.85 % and 21.29 % respectively.

Regarding the composition of the lipid fraction, we can observe a relatively high concentration of polyunsaturated fatty acids as well as the ω-3 and ω-6 fractions (Table 3). Carocho et al. [29] showed values of 4.8 ± 0.5 % for polyunsaturated fatty acids, somewhat inferior to the values obtained in this study (6.46 ± 1.35 %). Both omega-3 (ω-3) and omega-6 (ω-6) fatty acids are components of cell membranes, are precursors of many other substances in the body with functions such as the regulation of blood pressure and protection against fatal heart disease [31,32], they present anti-inflammatory effects [17], there is a growing interest in the role of omega-3 fatty acids in the prevention of diabetes [33] and certain types of cancer [18] and also in the prevention of mood and anxiety disorders [34].

In the SE cheese samples analysed, the polyunsaturated fatty acids α-linoleic acid (LA) and the α-linolenic acid (ALA) were found in the amount of 4.4 ± 1.12 % and 1.79 ± 0.51 %, respectively (table 3). In the human body, LA and ALA compete for metabolism by the enzyme delta-6-desaturase [35]. It has been suggested that this is important to health, as too high an intake of LA would reduce the amount of delta 6-desaturase available for the metabolism of ALA, which may increase the risk of heart disease.

Also, SE cheese samples presented a high content in short and medium chain fatty acids, namely C4:0, C6:0 and C:8, with an amount of 12.71 ± 2.11 % of the lipid fraction. These FA are directly associated with the characteristic flavour of SE cheese [4] and possess important biological properties in digestion, lipid metabolism and treatment of lipid malabsorption syndromes [36]. Carocho et al. [37] detected in their samples of SE cheese a slightly lower content of those SCFAs and MCFAs, a value around 7.5 %.

Fatty acids (% of the lipid fraction)	Serra Estrela cheese producers					
	Q1	Q2	Q3	Q4	Q5	Q6
Butyric acid C4:0	5.47±1.15^{a}	4.86±1.31^{a}	4.72±1.84^{a}	4.42±0.93^{a}	4.47±1.09^{a}	4.62±1.05^{a}
Caproic acid C6:0	5.14±0.44^{ab}	3.81±0.51^{a}	4.42±0.98^{ab}	4.27±0.36^{ab}	4.14±0.52^{ab}	5.20±0.20^{b}
Caprylic acid C8:0	4.52±0.79^{ab}	2.78±0.40^{a}	3.99±1.00^{ab}	3.82±0.74^{ab}	4.06±0.67^{ab}	5.25±0.87^{b}
Capric acid C10:0	11.08±2.11^{ab}	8.19±0.76^{a}	9.81±1.82a	11.43±2.44^{ab}	10.61±1.53^{ab}	14.30±1.01^{b}
Palmitoleic acid C16:1	0.881±0.055^{ab}	1.01±0.22^{b}	1.09±0.16^{b}	1.00±0.12b	0.900±0.167^{ab}	0.641±0.165^{a}
Oleic acid C18:1	4.78±1.57^{ab}	6.13±0.77^{ab}	7.88±1.31^{b}	5.59±0.64^{ab}	3.44±0.92^{a}	5.95±2.18^{ab}
α-linoleic acid (LA) C18:2	4.46±1.12^{a}	6.20±0.53^{a}	5.41±0.70^{a}	4.72±0.62^{a}	4.99±0.52^{a}	5.20±1.41^{a}
α-linolenic acid (ALA) C18:3	1.79±0.51^{a}	2.21±0.31^{a}	1.68±0.30^{a}	1.67±0.14^{a}	1.82±0.40^{a}	1.51±0.38^{a}
Eicosenoic acid C20:1	0.141±0.082^{a}	0.078±0.004^{a}	0.109±0.021^{a}	0.049±0.017^{a}	0.093±0.047^{a}	0.110±0.066^{a}
Arachidonic acid C20:4	0.211±0.171^{a}	0.434±0.182^{a}	0.346±0.282^{a}	0.182±0.175^{a}	0.336±0.094^{a}	0.455±0.148^{a}
Polyunsaturated fatty acids	6.46±1.35^{a}	8.84±0.70^{a}	7.44±1.09^{a}	6.56±0.82^{a}	7.15±0.85^{a}	6.70±1.93^{a}
Sum ω-3	1.79±0.52^{a}	2.21±0.31^{a}	1.68±0.30^{a}	1.67±0.14^{a}	1.82±0.40^{a}	1.16±0.77^{a}
Sum ω-6	4.67±1.13^{a}	6.63±0.41^{a}	5.76±0.79^{a}	4.90±0.72^{a}	5.33±0.46^{a}	5.54±1.52^{a}

Table 3: Composition in some fatty acids determined by GC-FID in 24 samples of Serra Estrela cheese, by cheese producer.

Values presented as mean ± standard deviation; for each parameter, values with different letters are significantly different (Tukey, $p < 0.05$); GC-FID: gas chromatography coupled with flame ionization detector; Q1-Q6: Serra Estrela cheese producers.

It was found that some SE cheese samples exceeded the maximum permissible values of microorganisms, in the determinations of *Escherichia coli* and *Staphylococcus* coagulase-positive (Figure 1).

Conclusion

In the Central Region of Portugal, Serra Estrela cheese plays an important role in the so called rural based economy namely for many

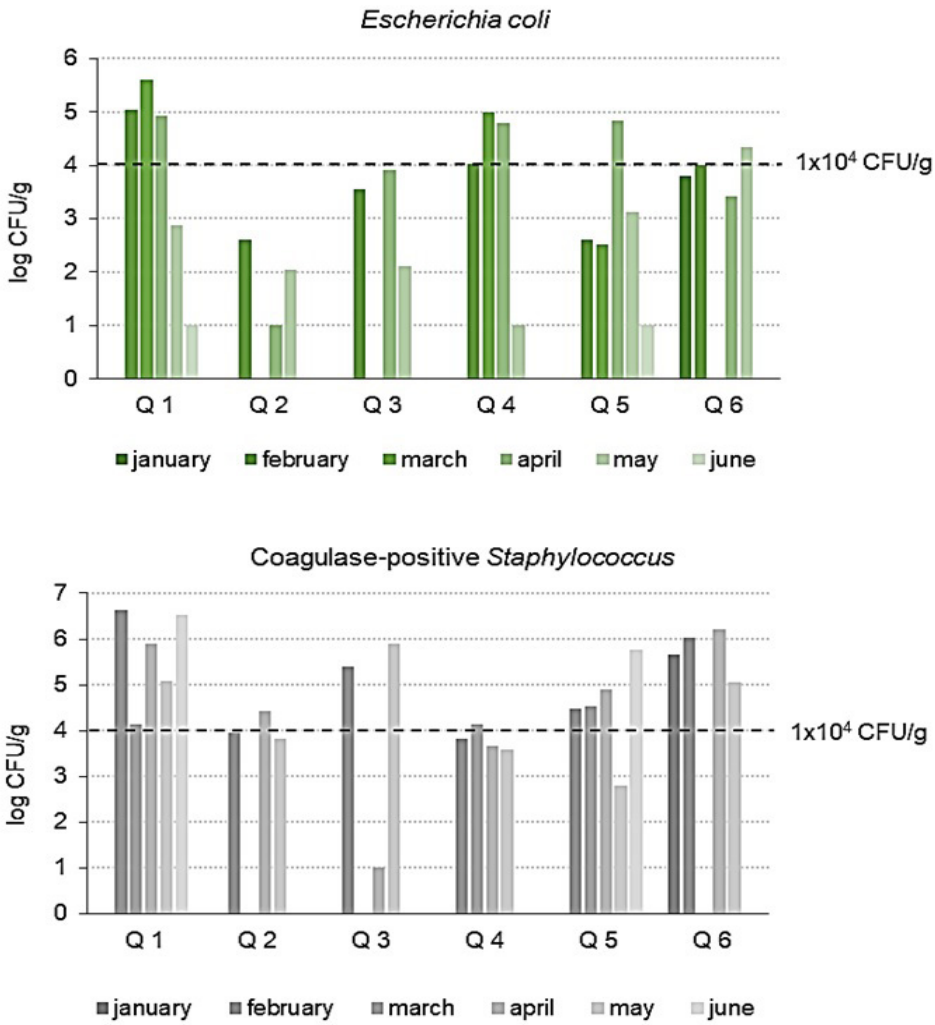

Figure 1: Escherichia coli and coagulase-positive Staphylococcus determinations in SE cheese samples.
SE: Serra Estrela; Q1-Q6: Serra Estrela cheese producers; CFU/g: colony forming units/gram of cheese; dashed line: maximum limit allowed.

families that survive thanks to cheese production and traditional products, resulting a unique nutritional and sensory element with characteristics that must be valued in terms of health promotion and well-being.

Regarding the obtained results in microbiological terms, the hygienic-sanitary quality of SE cheeses reveals that the artisanal manufacture can constitute nowadays a problem, revealing that the hygiene process (namely of the hands) in the several steps of production process may become a contamination factor. Nevertheless, the lactation period of 2017/2018 was considered an exception, due to the severe drought and to the catastrophic fires that devastated the region, with severe repercussions on health and animal production. It is also essential for the competent authorities to ensure that the current legislation in force is being complied to avoid possible consumer health problems when hygienic-sanitary shortcomings are evaluated.

Milk and dairy products are considered an important part of a balanced diet. Considering that the 2015–2020 Dietary Guidelines for Americans [38] state that fats should be between 25-35% of the total energy value, the future study of the reference dose becomes essential in order to demonstrate the possible benefits in consuming a small portion of SE cheese per week. Regarding the lipid fraction of SE cheese presents high values of short and medium fatty acids (SCFAs and MCFAs), namely butyric, capronic, caprylic and capric acids, medium concentrations of monounsaturated fatty acids (MUFAs) such as oleic, palmitoleic and eicosenoic acids and medium values of polyunsaturated fatty acids (PUFAs) with possible different functional properties such as α-linoleic acid, arachidonic acid and α-linolenic acid.

This project will broadcast to the producers of SE cheese the knowledge for respond to the variation of technological and sensorial aspects and for understanding SE cheese as a product with potential health benefits. The strategy is based on a synergy between a research centre, an exclusive product and its territories and landscapes, to create an extra value for the rural economy and promoting a sustainable environment.

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Competing Interests

The authors declare they have no competing interests.

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